In the Specification:

On page 1, please amend the "Related Applications" paragraph (lines 6-8) as follows:

This application is a divisional application of U.S. Application Serial No. 09/561,763, filed April 28, 2000, which is a continuation-in-part of ~~This application claims priority to~~ U.S. Patent Application Serial No.: 09/431,367, filed on November 1, 1999, which is a continuation-in-part of [[and]] U.S. Patent Application Serial No.: 09/259,951, filed on March 1, 1999, all of which are hereby incorporated herein by referenced in their entirety. ~~incorporated herein in their entirety by this reference.~~

At page 3, lines 29-35, please replace the text with the following paragraph:

In one embodiment, a TWIK nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a complement thereof.

At page 4, line 31 through page 6, line 6, please replace the text with the following paragraphs:

In another embodiment, a TWIK nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. In a preferred embodiment, a TWIK nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human TWIK-2, TWIK-3, TWIK-4, or TWIK-5. In yet another preferred embodiment, the nucleic acid molecule includes a nucleotide sequence encoding a protein having

the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, or SEQ ID NO:11, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. In yet another preferred embodiment, the nucleic acid molecule is at least 537 nucleotides in length and encodes a protein having a TWIK activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably TWIK nucleic acid molecules, which specifically detect TWIK nucleic acid molecules relative to nucleic acid molecules encoding non-TWIK proteins. For example, in one embodiment, such a nucleic acid molecule is at least 369, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:4, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-397, 586-670, 904-1111, or 1573-1575 of SEQ ID NO:4. In other preferred embodiments, the nucleic acid molecules comprise nucleotides 1-397, 586-670, 904-1111, or 1573-1575 of SEQ ID NO:4.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 537, 550-600, 600-650, 650-700, 700-750, 750-800 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:7, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a complement thereof. In preferred embodiments, the nucleic acid molecules are at least 15 (*e.g.*, contiguous) nucleotides in length and hybridize under stringent conditions to nucleotides 1-164, 207-404, 1037-1789, 1818-1869, 1972-1985, or 2258-2287 of SEQ ID NO:7. In other preferred embodiments, the nucleic acid molecules include nucleotides 1-164, 207-404, 1037-1789, 1818-1869, 1972-1985, or 2258-2287 of SEQ ID NO:7.

In another particularly preferred embodiment, the nucleic acid molecule comprises a fragment of at least 550-600, 600-650, 650-700, 700-750, 750-800, 805, 850-900 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:10, the nucleotide sequence of the

DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a complement thereof.

In other preferred embodiments, the nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, 5, 8, 11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, 3, 4, 6, 7, 9, 10, or 12 under stringent conditions.

At page 6, line 17 through page 7, line 12, please replace the text with the following paragraphs:

Another aspect of this invention features isolated or recombinant TWIK proteins and polypeptides. In one embodiment, the isolated protein, preferably a TWIK protein, includes at least one transmembrane domain. In another embodiment, the isolated protein, preferably a TWIK protein, includes at least one P-loop. In another embodiment, the isolated protein, preferably a TWIK protein, includes at least one transmembrane domain and at least one P-loop. In a preferred embodiment, the protein, preferably a TWIK protein, includes at least one transmembrane domain and at least one P-loop and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:11 or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. In another preferred embodiment, the protein, preferably a TWIK protein, includes at least one transmembrane domain and plays a role in generating an electrical potential across a plasma membrane, *e.g.*, a neuronal plasma membrane or a muscle plasma membrane. In another preferred embodiment, the protein, preferably a TWIK protein, includes at least one P-loop and plays a role in generating an electrical potential across a plasma membrane, *e.g.*, a neuronal plasma membrane or a muscle plasma membrane. In another preferred embodiment, the protein, preferably a TWIK protein, includes at least one transmembrane domain and at least one P-loop, and plays a role in generating an electrical potential across a plasma membrane, *e.g.*, a neuronal plasma membrane or a muscle plasma membrane. In yet another preferred embodiment, the protein, preferably a TWIK protein, includes at least one transmembrane domain and at least one P-loop and is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ

ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:10, or SEQ ID NO:12.

In another embodiment, the invention features fragments of the proteins having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, or SEQ ID NO:11, wherein the fragment comprises at least 15 amino acids (*e.g.*, contiguous amino acids) of the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with the ATCC as Accession Number PTA-1640. In another embodiment, the protein, preferably a TWIK protein, has the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11.

At page 11, line 22 through page 12, line 3, please replace the text with the following paragraph:

As used herein, a "potassium channel associated disorder" includes a disorder, disease or condition which is characterized by a misregulation of a potassium channel mediated activity. Potassium channel associated disorders can detrimentally affect conveyance of sensory impulses from the periphery to the brain and/or conductance of motor impulses from the brain to the periphery; integration of reflexes; interpretation of sensory impulses; and emotional, intellectual (*e.g.*, learning and memory), or motor processes. Examples of potassium channel associated disorders include CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy, and Jakob-Creutzfieldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; and neurological disorders, *e.g.*, migraine. Further examples of potassium channel associated disorders include obesity; cardiac disorders, *e.g.*, cardiac arrhythmias ~~arrythmias~~; and pain disorders, *e.g.*, pain disorders associated with various forms of tissue injury, such as inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-Hill), tooth pain, headaches (*e.g.*, tension headache or migraine), back pain, cancer pain, arthritis pain, psychogenic pain, pain associated with surgery, or neuropathic pain.

At page 12, lines 21-36, please replace the text with the following paragraph:

For example, the family of TWIK proteins comprise at least one "transmembrane domain" and preferably four transmembrane domains. As used herein, the term "transmembrane domain" includes an amino acid sequence of about 15 amino acid residues in length which spans the plasma membrane. More preferably, a transmembrane domain includes about at least 20, 25, 30, 35, 40, or 45 amino acid residues and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, and typically have an α-helical structure. In a preferred embodiment, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains are described in, for example, Zagotta W.N. ~~et al.~~ *et al.* (1996) *Annu. ~~Annual~~ Rev. Neurosci. ~~Neuronsci.~~* 19: 235-63, the contents of which are incorporated herein by reference. Amino acid residues 7-23, 113-134, 160-184, and 225-242 of the human TWIK-2 protein, amino acid residues 23-43, 131-148, 178-200, and 244-264 of the human TWIK-3 protein, amino acid residues 7-26, 121-140, 169-193, and 236-253 of the human TWIK-4 protein comprise transmembrane domains, and amino acid residues 37-61, 148-165, 298-321, and 353-372 of the human TWIK-5 protein comprise transmembrane domains.

At page 12, line 37 through page 13, line 15, please replace the text with the following paragraph:

In another embodiment, a TWIK molecule of the present invention is identified based on the presence of a P-loop. As used herein, the term "P-loop" (also known as an H5 domain) includes an amino acid sequence of about 15-45 amino acid residues in length, preferably about 15-35 amino acid residues in length, and most preferably about 15-25 amino acid residues in length, which is involved in lining the potassium channel pore. A P-loop is typically found between transmembrane domains of potassium channels and is believed to be a major determinant of ion selectivity in potassium channels. Preferably, P-loops contain a G-[HYDROPHOBIC AMINO ACID]-G sequence, *e.g.*, a GYG, GLG, or GFG sequence. P-loops are described in, for example, Warmke *et al.* (1991) *Science* 252:1560-1562; Zagotta W.N. *et al.*, (1996) *Annu. ~~Annual~~ Rev. Neurosci. ~~Neuronsci.~~* 19:235-63 [[(]]Pongs, O. (1993) *J. Membr. Biol.*, 136, 1-8; Heginbotham *et al.* (1994) *Biophys. J.* 66,1061-1067; Mackinnon, R. (1995) *Neuron*, [[and]] 14, 889-892; Pascual *et al.*, (1995) *Neuron.*, 14, 1055-1063[[)]], the contents of which are incorporated herein by reference. Amino acid residues 88-105 and 194-213 of the human

TWIK-2 protein, amino acid residues 103-119 and 212-229 of the human TWIK-3 protein, amino acid residues 93-109 and 204-221 of the human TWIK-4 protein comprise a P-loop, and amino acid residues 118-139 and 328-345 of the human TWIK-5 protein comprise a P-loop.

At page 14, line 33 through page 15, line 33, please replace the text with the following paragraphs:

The nucleotide sequence of the isolated human TWIK-2 cDNA and the predicted amino acid sequence of the human TWIK-2 polypeptide are shown in Figure 1 and in SEQ ID NOs:1 and 2, respectively. ~~A plasmid containing the nucleotide sequence encoding human TWIK-2 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.~~

The human TWIK-2 gene, which is approximately 3452 nucleotides in length, encodes a protein having a molecular weight of approximately 57.4 kD and which is approximately 499 amino acid residues in length.

The nucleotide sequence of the isolated human TWIK-3 cDNA and the predicted amino acid sequence of the human TWIK-3 polypeptide are shown in Figure 3 and in SEQ ID NOs:4 and 5, respectively. ~~A plasmid containing the nucleotide sequence encoding human TWIK-3 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.~~

The human TWIK-3 gene, which is approximately 1575 nucleotides in length, encodes a protein having a molecular weight of approximately 38.2 kD and which is approximately 332 amino acid residues in length.

The nucleotide sequence of the isolated human TWIK-4 cDNA and the predicted amino acid sequence of the human TWIK-4 polypeptide are shown in Figure 5 and in SEQ ID NOs:7

and 9, respectively. ~~A plasmid containing the nucleotide sequence encoding human TWIK-4 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.~~

The human TWIK-4 gene, which is approximately 2287 nucleotides in length, encodes a protein having a molecular weight of approximately 36 kD and which is approximately 313 amino acid residues in length.

The nucleotide sequence of the isolated human TWIK-5 cDNA and the predicted amino acid sequence of the human TWIK-5 polypeptide are shown in Figure 17 and in SEQ ID NOs:10 and 11, respectively. A plasmid containing the nucleotide sequence encoding human TWIK-5 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on April5, 2000 and assigned Accession Number PTA-1640. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

At page 16, line 25 through page 17, line 8, please replace the text with the following paragraphs:

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, as a hybridization probe, TWIK nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in

Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640.

At page 18, line 7 through page 20, line 24, please replace the text with the following paragraphs:

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, such that it can hybridize to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule of the present invention comprises a nucleotide sequence which is at least about 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, 90%, 95% or more identical to the entire length of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the entire length of the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a portion of any of these nucleotide sequences.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, for example a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a TWIK protein. The nucleotide sequence determined from the cloning of the TWIK gene allows for the generation of probes and primers designed for use in identifying and/or cloning other TWIK family members, as well as TWIK homologues from other species.

The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, of an anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. In an exemplary embodiment, a nucleic acid molecule of the present invention comprises a nucleotide sequence which is 369, 350-400, 400-450, 450-500, 500-550, 537, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 949, 950-1000, 1575, 2287, or 3452 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the

nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640.

Probes based on the TWIK nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or identical proteins. In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a TWIK protein, such as by measuring a level of a TWIK-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting TWIK mRNA levels or determining whether a genomic TWIK gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of a TWIK protein" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, which encodes a polypeptide having a TWIK biological activity (the biological activities of the TWIK proteins are described herein), expressing the encoded portion of the TWIK protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the TWIK protein.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, due to degeneracy of the genetic code and thus encode the same TWIK proteins as those encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11.

In addition to the TWIK nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession

Number PTA-1640, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the TWIK proteins may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the TWIK genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a TWIK protein, preferably a mammalian TWIK protein, and can further include non-coding regulatory sequences, and introns.

At page 21, line 7 through page 23, line 27, please replace the text with the following paragraphs:

Moreover, nucleic acid molecules encoding other TWIK family members and, thus, which have a nucleotide sequence which differs from the TWIK sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640 are intended to be within the scope of the invention. For example, another TWIK cDNA can be identified based on the nucleotide sequence of human TWIK. Moreover, nucleic acid molecules encoding TWIK proteins from different species, and thus which have a nucleotide sequence which differs from the TWIK sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640 are intended to be within the scope of the invention. For example, a mouse TWIK cDNA can be identified based on the nucleotide sequence of a human TWIK.

Nucleic acid molecules corresponding to natural allelic variants and homologues of the TWIK cDNAs of the invention can be isolated based on their homology to the TWIK nucleic acids disclosed herein using the cDNAs disclosed herein, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as

Accession Number PTA-1640. In other embodiment, the nucleic acid is at least 30, 50, 100, 150, 200, 250, 300, 307, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 949, or 950 nucleotides in length. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% identical to each other typically remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50°C, preferably at 55°C, and more preferably at 60°C or 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the TWIK sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, thereby leading to changes in the amino acid sequence of the encoded TWIK proteins, without altering the functional ability of the TWIK proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TWIK (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the TWIK proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved

between the TWIK proteins of the present invention and other members of the TWIK potassium channel families are not likely to be amenable to alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding TWIK proteins that contain changes in amino acid residues that are not essential for activity. Such TWIK proteins differ in amino acid sequence from SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more identical to SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11.

An isolated nucleic acid molecule encoding a TWIK protein identical to the protein of SEQ ID NO: 2, SEQ ID NO:5, SEQ ID NO:8, or SEQ ID NO:11 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced into SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a TWIK protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a TWIK coding sequence, such as by saturation

mutagenesis, and the resultant mutants can be screened for TWIK biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

At page 25, line 30 through page 26, line 8, please replace the text with the following paragraph:

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in ~~Haselhoff~~ Haseloff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TWIK mRNA transcripts to thereby inhibit translation of TWIK mRNA. A ribozyme having specificity for a TWIK-encoding nucleic acid can be designed based upon the nucleotide sequence of a TWIK cDNA disclosed herein (*i.e.*, SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TWIK-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, TWIK mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

At page 29, line 35 through page 30, line 23, please replace the text with the following paragraphs:

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at [[http://]]www.gcg.com), using either a

Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at [[http://]]www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to TWIK nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to TWIK protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See [[http://]]www.ncbi.nlm.nih.gov.

At page 33, lines 1-13, please replace the text with the following paragraph:

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TWIK proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis

(REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify TWIK variants (Arkin and Youvan ~~Yourvan~~ (1992) *Proc. Natl. Acad. Sci. USA* ~~89:7811-7815; Delgrave~~ 89:7811-7815; Delgrave *et al.* (1993) *Protein Eng.* ~~*Engineering*~~ 6(3):327-331).

At page 35, line 24 through page 36, line 23, please replace the text with the following paragraphs:

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-TWIK antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with TWIK to thereby isolate immunoglobulin library members that bind TWIK. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No. 5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT International Publication No. WO 91/17271; Winter *et al.* PCT International Publication No. WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling *et al.* PCT International Publication No. WO 93/01288; McCafferty *et al.* PCT International Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO 92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.* (1991) *Bio/Technology* 9:~~1370~~ 1369-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; ~~Clarkson~~ Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; ~~Garrad~~ Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) ~~*Nuc.*~~ *Nucleic Acid Res.* 19:4133-4137; Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.* ~~*Nature*~~ (1990) Nature 348:552-554.

Additionally, recombinant anti-TWIK antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA

techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application No. 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application No. 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) ~~*Canc.*~~ *Cancer Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; ~~Verhoeyan~~ Verhoeyen *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

At page 39, lines 15-19, please replace the text with the following paragraph:

In another embodiment, the TWIK expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) ~~*Embo*~~ *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (~~InVitrogen~~ Invitrogen Corporation, San Diego, CA).

At page 42, lines 7-31, please replace the text with the following paragraph:

A transgenic animal of the invention can be created by introducing a TWIK-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The TWIK cDNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of a human TWIK gene, such as a mouse or rat TWIK gene, can be used as a transgene. Alternatively, a TWIK gene homologue, such as another TWIK potassium channel family member, can be isolated based on hybridization to the TWIK cDNA sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12 or the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640 (described further in subsection I above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in

the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a TWIK transgene to direct expression of a TWIK protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of a TWIK transgene in its genome and/or expression of TWIK mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a TWIK protein can further be bred to other transgenic animals carrying other transgenes.

At page 45, lines 4-22, please replace the text with the following paragraph:

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy ~~syringability~~ syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable

compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

At page 49, line 27 through page 50, line 8, please replace the text with the following paragraph:

The isolated nucleic acid molecules of the invention can be used, for example, to express TWIK protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect TWIK mRNA (*e.g.*, in a biological sample) or a genetic alteration in a TWIK gene, and to modulate TWIK activity, as described further below. The TWIK proteins can be used to treat disorders characterized by insufficient or excessive production of a TWIK substrate or production of TWIK inhibitors. In addition, the TWIK proteins can be used to screen for naturally occurring TWIK substrates, to screen for drugs or compounds which modulate TWIK activity, as well as to treat disorders characterized by insufficient or excessive production of TWIK protein or production of TWIK protein forms which have decreased or aberrant activity compared to TWIK wild type protein (*e.g.*, CNS disorders such as neurodegenerative disorders, *e.g.*, Alzheimer's disease, dementias related to Alzheimer's disease (such as Pick's disease), Parkinson's and other Lewy diffuse body diseases, multiple sclerosis, amyotrophic lateral sclerosis, progressive supranuclear palsy, epilepsy and Jakob-Creutzfieldt disease; psychiatric disorders, *e.g.*, depression, schizophrenic disorders, korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, *e.g.*, amnesia or age-related memory loss; neurological disorders, *e.g.*, migraine; obesity; and cardiac disorders, *e.g.*, cardiac arrhythmia ~~arrythmia~~). Moreover, the anti-TWIK antibodies of the invention can be used to detect and isolate TWIK proteins, regulate the bioavailability of TWIK proteins, and modulate TWIK activity.

At page 51, lines 5-19, please replace the text with the following paragraph:

In one embodiment, an assay is a cell-based assay in which a cell which expresses a TWIK protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate TWIK activity is determined. Determining the ability of the test compound to modulate TWIK activity can be accomplished by monitoring, for example, the release of a neurotransmitter form a cell which expresses TWIK. The cell, for example, can be of mammalian origin. Determining the ability of the test compound to modulate the ability of TWIK to bind to a substrate can be accomplished, for example, by coupling the

TWIK substrate with a radioisotope or enzymatic label such that binding of the TWIK substrate to TWIK can be determined by detecting the labeled TWIK substrate in a complex. For example, compounds (*e.g.*, TWIK substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radio emission ~~radioemmission~~ or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

At page 59, line 27 through page 60, line 2, please replace the text with the following paragraph:

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining TWIK protein and/or nucleic acid expression as well as TWIK activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant TWIK expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with TWIK protein, nucleic acid expression or activity. For example, mutations in a TWIK gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby ~~phophylactically~~ prophylactically treat an individual prior to the onset of a disorder characterized by or associated with TWIK protein, nucleic acid expression or activity.

At page 60, lines 8-21, please replace the text with the following paragraph:

An exemplary method for detecting the presence or absence of TWIK protein or nucleic acid in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting TWIK protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes TWIK protein such that the presence of TWIK protein or nucleic acid is detected in the biological sample. A preferred agent for detecting TWIK mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to TWIK mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length TWIK nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:3, SEQ ID

NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:12, or the DNA insert of the plasmid deposited with ATCC as Accession Number PTA-1640, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to TWIK mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

At page 72, line 26 through page 73, line 16, please replace the text with the following paragraphs:

The nucleotide sequence encoding the human TWIK-2 protein is shown in Figure 1 and is set forth as SEQ ID NO:1. The full length protein encoded by this nucleic acid comprises about 499 amino acids and has the amino acid sequence shown in Figure 1 and set forth as SEQ ID NO:2. The coding region (open reading frame) of SEQ ID NO:1 is set forth as SEQ ID NO:3. ~~Clone Fthka20g4, comprising the entire coding region of human TWIK-2 was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.~~

The nucleotide sequence encoding the human TWIK-3 protein is shown in Figure 3 and is set forth as SEQ ID NO:4. The full length protein encoded by this nucleic acid comprises about 332 amino acids and has the amino acid sequence shown in Figure 3 and set forth as SEQ ID NO:5. The coding region (open reading frame) of SEQ ID NO:4 is set forth as SEQ ID NO:6. ~~Clone Athua133f10, comprising the entire coding region of human TWIK-3 was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.~~

The nucleotide sequence encoding the human TWIK-4 protein is shown in Figure 5 and is set forth as SEQ ID NO:7. The full length protein encoded by this nucleic acid comprises about 313 amino acids and has the amino acid sequence shown in Figure 5 and set forth as SEQ ID NO:8. The coding region (open reading frame) of SEQ ID NO:7 is set forth as SEQ ID NO:9. ~~Clone AthTb005e07, comprising the entire coding region of human TWIK-4 was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on _____, and assigned Accession No. _____.~~

The nucleotide sequence encoding the human TWIK-5 protein is shown in Figure 17 and is set forth as SEQ ID NO:10. The full length protein encoded by this nucleic acid

comprises about 401 amino acids and has the amino acid sequence shown in Figure 17 and set forth as SEQ ID NO:11. The coding region (open reading frame) of SEQ ID NO:10 is set forth as SEQ ID NO:12. Clone Fbh51164a, comprising the entire coding region of human TWIK-5 was deposited with the American Type Culture Collection (ATCC®), 10801 University Boulevard, Manassas, VA 20110-2209, on April 5, 2000, and assigned Accession No. PTA-1640.

At page 75, lines 7-16, please replace the text with the following paragraphs:

The TWIK-2 protein was aligned with the hTASK-2 protein (described in Reyes ~~Reyer~~ R. *et al.* (1998) *J. Biol. Chem.* 273(47):30863-30869) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4. The results showed a 100% identity and 100% similarity between the two protein sequences (see Figure 16).

The TWIK-5 protein was aligned with the hTASK-2 protein (described in Reyes ~~Reyer~~ R. *et al.* (1998) *J. Biol. Chem.* 273(47):30863-30869) using the GAP program in the GCG software package (PAM250 matrix) and a gap weight of 25 and a length weight of 1. The results showed a 29.183% identity and 49.805% similarity between the two protein sequences (see Figure 19).

At page 78, line 32 through page 79, line 3, please replace the text with the following paragraph:

To electrophysiologically characterize the TWIK-5 molecule, the full length human TWIK-5 gene in the pMet7 expression vector was used to transiently transfect CHO cells using lipofectamine. ~~[TO INVENTORS: PLEASE CONFIRM TRANSFECTION METHODOLOGIES]~~ Electrophysiological measurements in the transfected CHO cells were taken using a single electrode patch-clamp, 48 hours after transfection. Cell membrane potentials were held at –80mV and then depolarized from –60 mV to +50 mV with 10 mV increments for 50 msec, followed by 20 msec hyperpolarization to –120 msec (see Figure 21A, lower panel). TWIK-5 displayed outward currents from –60 to +50 mV (Figure 21A, upper panel). The amplitude of current at +50 mV was 4 mA. The current-membrane potential curve was linear from +10mV to +50 mV (Figure 21B).